Attorney Docket No. 5455.210-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Widner *et al.*

Serial No.: To be assigned Group Art Unit: To be assigned

Filed: April 12, 2001 Examiner: To be assigned

For: Methods For Producing a Polypeptide in a Bacillus Cell

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Before the above-captioned application is taken up for examination, entry of the following amendment is respectfully requested (a marked up version pursuant to 37 C.F.R. 1.21 is attached hereto):

IN THE SPECIFICATION

At page 1, amend lines 6-7 to read as follows:

--This application is a divisional of pending U.S. application serial no. 09/256,377 filed on February 26, 1999, which is a continuation-in-part of U.S. Patent No. 5,955,310 issued on September 21, 1999, which are fully incorporated herein by reference.--

IN THE CLAIMS

Please cancel claims 1-73 without prejudice or disclaimer. Please add new claims 74-93:

74. A *Bacillus* cell comprising a nucleic acid construct which comprises (a) a "consensus" promoter having the sequence TTGACA for the "- 35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (b) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide.

75. The *Bacillus* cell of claim 74, wherein the consensus promoter is obtained from any

bacterial promoter.

76. The *Bacillus* cell of claim 75, wherein the "consensus" promoter is obtained from a *Bacillus* promoter.

77. The *Bacillus* cell of claim 74, wherein the consensus promoter is obtained from a promoter obtained from the *E. coli lac* operon, *Streptomyces coelicolor* agarase gene (*dagA*), *Bacillus lentus* alkaline protease gene (*aprH*), *Bacillus licheniformis* alkaline protease gene (subtilisin Carlsberg gene), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus* subtilis alpha-amylase gene (*amyE*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus subtilis xylA* and *xylB* genes, *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIA gene (*cryIIIA*, SEQ ID NO. 21), or prokaryotic beta-lactamase gene *spo1* bacterial phage promoter.

78. The *Bacillus* cell of claim 74, wherein the "consensus" promoter is obtained from the *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*).

79. The *Bacillus* cell of claim 78, wherein the "consensus" *amyQ* promoter has the nucleic acid sequence of SEQ ID NO. 26 or SEQ ID NO. 27.

80. The *Bacillus* cell of claim 74, wherein the mRNA processing/stabilizing sequence is the *cryIIIA* mRNA processing/stabilizing sequence.

81. The *Bacillus* cell of claim 74, wherein the mRNA processing/stabilizing sequence is the SP82 mRNA processing/stabilizing sequence.

82. The *Bacillus* cell of claim 74, which contains one or more copies of the nucleic acid construct.

83. The *Bacillus* cell of claim 74, which contains one copy of the nucleic acid construct.

84. The *Bacillus* cell of claim 74, wherein the nucleic acid construct further comprises a selectable marker gene.

85. The *Bacillus* cell of claim 74, which contains no selectable marker gene.

86. The *Bacillus* cell of claim 74, wherein the nucleic acid sequence encodes a polypeptide heterologous to the *Bacillus* cell.

87. The *Bacillus* cell of claim 74, wherein the polypeptide is a hormone, enzyme, receptor, antibody, or reporter.

88. The *Bacillus* cell of claim 87, wherein the enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase.

89. The *Bacillus* cell of claim 87, wherein the enzyme is an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, or xylanase.

90. The *Bacillus* cell of claim 74, wherein the nucleic acid sequence is contained in the chromosome of the *Bacillus* cell.

91. The *Bacillus* cell of claim 74, wherein the nucleic acid sequence is contained on an extrachromosomal element.

92. The *Bacillus* cell of claim 74, which is a *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*, *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or *Bacillus thuringiensis* cell.

93. The *Bacillus* cell of claim 74, which *Bacillus subtilis* cell.

REMARKS

This application is a divisional of pending U.S. application serial no. 09/256,377 filed on February 26, 1999, which is a continuation-in-part of U.S. Patent No. 5,955,310 issued on

September 21, 1999, which application and patent are fully incorporated herein by reference.

Claims 1-73 have been cancelled and new claims 74-93 added.

Respectfully submitted,

Date: April 12, 2001

Robert L. Starnes, Reg. No. 41,324
Novozymes Biotech, Inc.
1445 Drew Avenue
Davis, CA 95616
(530) 757-8100

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VERSION WITH MARKINGS TO SHOW CHANGES MADE UNDER 37 C.F.R. 1.21

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Below is a marked-up version of the amendment made in the accompanying amendment.

IN THE SPECIFICATION:

At page 1, amend lines 6-7 to read as follows:

Cross-Reference to Related Applications

~~This application is a continuation-in-part of application serial no. 09/031,442 filed February 26, 1998, the contents of which are fully incorporated herein by reference.~~ This application is a divisional of pending U.S. application serial no. 09/256,377 filed on February 26, 1999, which is a continuation-in-part of U.S. Patent No. 5,955,310 issued on September 21, 1999, which are fully incorporated herein by reference.

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